Environmental Health Perspectives
Vol. 95, pp. 199–204, 1991

Are Genotoxic Carcinogens More Potent Than Nongenotoxic Carcinogens?

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In this report we have raised the question whether genotoxic carcinogens are more potent than nongenotoxic carcinogens when studied in long-term carcinogenicity assays in rodents. To build a large database of compounds for which both carcinogenicity and genotoxicity had been investigated, we have used a database produced by Gold and co-workers for carcinogenic potency data (975 chemicals) and a database produced by Würgler for genotoxicity data (2834 chemicals). Considering compounds positive or negative in at least three short-term tests and in at least 75% of available tests, we could define 67 genotoxic carcinogens and 46 nongenotoxic carcinogens. Carcinogenic potency of genotoxic carcinogens was about 50 times higher than carcinogenic potency of nongenotoxic carcinogens. Our results are different from the results of Tennant et al.; their database (24 genotoxic carcinogens and 12 nongenotoxic carcinogens compatible with our definition) seems to suggest that there is practically no difference in potency between genotoxic and nongenotoxic carcinogens. The two databases have only four compounds in common and are also different in terms of number of elements for different chemical classes. Nitrosocompounds, nitrogen mustards, hydrazine derivatives, and polycyclic aromatic hydrocarbons are not represented in the database of Tennant. The overall impression from our analysis is that the usefulness of short-term tests of genotoxicity could be significantly better than what has been suggested by the previous work of Tennant et al. because these tests tend to detect, at least for many important chemical classes, the most potent carcinogens. This consideration may not be valid for certain classes of chemicals.

Introduction

In a recent work (*1*), the capability of short-term tests in predicting carcinogenicity has been found to be much more limited than the estimates of previous assessments (*2,3*). Using equilibrated databases with similar numbers of genotoxic and nongenotoxic chemicals, assayed in short-term tests, and similar numbers of carcinogenic and noncarcinogenic chemicals assayed in rodent experiments, we could expect a 50% agreement of the two types of results just by chance. The actual level of agreement observed by Tennant et al. in their study (*1*) was only approximately 60%.

In this work we wanted to investigate a different aspect of the relationship between genotoxicity and carcinogenicity. We thus asked the question: Are genotoxic carcinogens, on average, more potent and therefore more dangerous than nongenotoxic ones? As short-term tests for epigenetic and/or promoting activities are currently not available, we could expect that if carcinogenicity is a function of both genotoxicity and epigenetic-promoting activities, chemicals evaluated as genotoxic can have both activities, while chemicals evaluated as nongenotoxic can have at most have only one. Therefore, genotoxic chemicals, from the point of view of potential carcinogenicity, start with a kind of advantage in respect to nongenotoxic agents.

For both carcinogenicity and genotoxicity we have tried using a larger database to obtain a reasonably large intersection database between carcinogenicity studies and genotoxicity studies. We are aware that literature-based evaluations can be subjected to potential bias in favor of either clearly genotoxic or clearly carcinogenic compounds because it is sometimes easier to publish positive rather than negative results. We analyze this problem after presenting our results.

Methods and Results

As a database for carcinogenic potency, we used that of Gold et al. (*4–6*). In this database, 975 chemicals are described, and 492 are defined as carcinogens (*8*) because tumor incidence in treated animals was found to be significantly higher than in control animals (in at least one target tissue or all tissues together), according to the conclusions of the authors of the experimental work. The other 483 chemicals can be defined as doubtful or negative and were not used in our study. As a database for genotoxicity, we used Würgler's database (*7*). In this database, 2834 chemicals and 95 different types of results are reported. Among them, 76 types of genotoxicity tests are considered; they can be subdivided in the following categories: repair tests, bacterial mutation assays, fungal assays, Drosophila assays, *in vitro* and *in vivo* mammalian assays. We used only genotoxicity

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Table 1. Intersection database from Gold et al. (8) and Würgler (7): chemicals positive or negative in at least three tests and in at least 75% of available tests.

	CAS number	Chemical name	$Log_{10}(TD_{50})$[a]
Genotoxic chemicals			
*[b]	50-00-0	Formaldehyde	−0.10
*	50-07-7	Mitomycin-C	−3.01
*	50-18-0	Cyclophosphamide	0.10
*	51-75-2	Nitrogen mustard	−1.94
*	52-24-4	Thio-tepa [*tris*(1-aziridinyl)phosphine sulfide)	−0.91
	53-70-3	Dibenz[*a*,*h*]anthracene	0.77
*	53-95-2	*N*-Hydroxy-2-acetylaminofluorene	−3.16
*	55-18-5	*N*-Nitrosodiethylamine	−2.10
	57-39-6	Metepa	0.65
*	57-57-8	β-Propiolactone	0.06
*	57-97-6	7,12-Dimethylbenz[*a*]anthracene	−1.08
*	62-75-9	*N*-Nitrosodimethylamine	−1.23
*	66-27-3	Methyl methanesulfonate	1.50
*	68-76-8	Trenimon	−2.30
*	70-25-7	*N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine	−0.28
*	75-09-2	Methylene chloride	2.78
*	75-21-8	Ethylene oxide	0.87
	79-34-5	1,1,2,2-Tetrachloroethane	1.55
*	79-44-7	Dimethylcarbamil chloride	0.73
*	96-12-8	1,2-Dibromo-3-chloropropane	−0.97
*	100-75-4	*N*-Nitrosopiperidine	0.11
*	100-93-4	1,2-Dibromoethane	0.04
*	107-06-2	1,2-Dichloroethane	0.74
*	107-13-1	Acrylonitrile	0.73
	109-84-2	2-Hydroxyethylhydrazine	−0.50
*	115-02-6	Azaserine	−0.10
*	117-39-5	Quercetin	0.71
*	126-72-7	Tris(2,3-dibromopropyl)phosphate	0.20
	127-18-4	Tetrachloroethylene	1.88
*	140-79-4	Dinitrosopiperazine	0.30
*	148-82-3	Melphalan	−1.14
*	151-56-4	Ethylene imine	−0.55
	305-03-3	Chlorambucil	−1.01
*	315-22-0	Monocrotaline	−0.10
*	512-56-1	Trimethylphosphate	2.53
	531-82-8	*N*-4-(5-Nitro-2-furyl)-2-thiazolylacetamide	1.02
	602-87-9	5-Nitroacenaphtene	0.78
*	614-95-9	Nitrosoethylurethan	−0.61
*	621-64-7	*N*-Nitrosodipropylamine	−0.73
	758-17-8	*N*-Methyl-*N*-formylhydrazine	−0.13
*	759-73-9	1-Ethyl-1-nitrosourea	−0.04
*	869-01-2	*N*-*N*-Butyl-*N*-nitrosourea	−0.04
	924-16-3	Nitrosodibutylamine	−0.16
	930-55-2	*N*-Nitrosopyrrolidine	0.19
	1068-57-1	Monoacetyl hydrazine	0.65
*	1120-71-4	Propane sultone	0.56
*	1162-65-8	Aflatoxin B_1	−3.03
	2318-18-5	Senkirkine	0.23
	3544-23-8	3-Methoxy-4-aminoazobenzene	1.78
	3570-75-0	Formic acid 2-4-(5-nitro-2-furyl)-2-thiazolyl hydrazide	0.55
*	3688-53-7	2-(2-Furyl)-3-(5-nitro-2-(furyl)acrylamide	1.06
	5307-14-2	2-Nitro-*p*-phenylenediamine	2.79
*	7227-91-0	1-Phenyl-3,3-dimethylatriazene	0.36
*	18883-66-4	Streptozotocin	−0.71
	22571-95-5	Symphytine	0.28
*	24554-26-5	*N*-4(5-Nitro-2-furyl)-2-thiazolylformamide	0.12
	25843-45-2	Azoxymethane	−1.52
	26049-69-4	2-(2,2-Dimethylhydrazino)-4-(5-nitro-2-furyl) thiazole	−0.39
	26148-68-5	2-Amino-9H-pyrido(2,3-*b*) indole	1.55
	28754-68-9	*trans*-5-Amino-3 2-(5-nitro-2-furyl) vinyl-1,2,4-oxadiazole	2.02
	38777-13-8	Nitroso-baygon	−0.44
	42011-48-3	2,2,2-Trifluoro-*N*-4-(5-nitro-2-furyl)-2-thiazolylacetamide	0.83
	54749-90-5	Chlorozotocin	−1.62
	67730-10-3	2-Aminodipyrido 1,2-*a*:3',2'-*d*-imidazole	1.08
	67730-11-4	2-Amino-6-methyldipyrido- 1,2-*a*:3',2'-*d*-imidazole	0.51
	68006-83-7	2-Amino-3-methyl-9H-pyrido- 2,3-*b* -indole	1.19
	76180-96-6	2-Amino-3-methylimidazo- 4,5-f quinoline	1.24
Nongenotoxic chemicals			
*[b]	50-06-6	Phenobarbital	0.62
*	56-53-1	Diethylstilbestrol	−1.59
*	57-14-7	1,1-Dimethylhydrazine	0.32
*	57-30-7	Phenobarbital sodium	1.54
*	60-34-4	Methylhydrazine	0.66
*	60-35-5	Acetamide	2.02
*	60-57-1	Dieldrin	−0.33
*	60-80-0	Phenazone	3.09
*	61-82-5	3-Aminotriazole	0.94
*	62-55-5	Thioacetamide	0.73
*	62-56-6	Thiourea	1.97
*	63-25-2	Carbaryl	1.15
*	64-17-5	Ethyl alcohol	3.96
*	67-72-1	Hexachloroethane	2.50
*	72-55-9	*p*,*p'*-DDE	0.98
*	76-44-8	Heptachlor	0.04
*	82-68-8	Pentachloronitrobenzene	1.85
*	86-30-6	*N*-Nitrosodiphenylamine	2.06
*	88-06-2	2,4,6-Trichlorophenol	2.61
*	88-19-7	*o*-Toluenesulfonamide	3.60
	94-58-6	Dihydrosafrole	1.95
*	95-06-7	Sulfallate	1.24
*	102-71-6	Triethanolamine	2.00
*	108-05-4	Vinyl acetate	2.12
*	117-81-7	Di(2-ethylhexyl)phthalate	3.36
*	128-37-0	Butylated hydroxytoluene	2.57
*	128-44-9	Saccharin sodium	3.05
*	309-00-2	Aldrin	−0.13
*	518-75-2	Citrinin	0.72
*	569-61-9	*p*-Rosaniline HCl	1.33
*	842-07-9	1-Phenylazo-2-naphthol	1.25
*	915-67-3	FD&C red no. 2	2.80
*	1582-09-8	Trifluralin	2.52
*	1694-09-3	FD&C violet no. 1	2.62
*	2303-16-4	Diallate	1.29
*	3564-09-8	FD&C red no. 1	2.35
	3761-53-3	FD&C red no. 5	2.37
*	4548-53-2	FD&C red no. 4	3.79
*	5141-20-8	FD&C green no. 2	3.75
*	5208-87-7	1'-Hydroxysafrole	1.08
*	13073-35-3	Ethionine	0.70
*	17924-92-4	Zearalenone	1.34
*	21884-44-6	Luteoskyrin	1.17
*	25013-16-5	Butylated hydroxyanisole	2.54
	51410-44-7	1'-Hydroxyestragole	1.76
	56222-35-6	*N*-Nitroso-3-hydroxypyrrolidine	0.88

[a] TD_{50} is defined as "that chronic dose rate (in mg/kg body weight/day) which would halve the actuarially adjusted percentage of tumor free animals at the end of a standard experiment time (the 'standard lifespan' for the species)" (*9*). When TD_{50} values both for mice and rats were available in the summary database of Gold et al. (*8*), the lower value was used. In that database, the judgment about the positivity of results is left to the authors of the experimental work. For a very small number of chemicals, Gold points out that the overall statistical significance of the results seems questionable. In this case we have accepted as positive only chemicals for which we could find, at least for a specific tissue, a statistical significance with $p < 0.05$, two-tailed, in the detailed databases of Gold et al. (*4–6*).

[b] The asterisk indicates that the compound was positive or negative in at least six tests and at least 75% of available tests.

data, discarding other types of information concerning carcinogenicity, promoting activity, plant systems and pooled data. The results were treated by Würgler (*7*) with a qualitative approach and defined as clearly positive, clearly negative, and questionable results. We have not considered questionable results in our study.

We have directly examined nearly all the publications that are sources for Würgler's database. From our analysis, approximately three out of four short-term tests were performed *in vitro* and approximately 25% were performed *in vivo*. For the tests performed *in vitro*, an acceptable metabolic activation was present in about 50% of the cases.

As a consequence, we have accepted a situation in which approximately 37% of the totality (*in vitro* + *in vivo*) of the tests performed is without metabolic activation. Most likely, because of this fact, some genotoxic compounds became doubtful compounds and some doubtful compounds became nongenotoxic ones. However, the relative difference between the two subclasses should have remained substantially the same.

The intersection of the databases of Gold et al. and Würgler made it possible to build a database about three times larger than that of Tennant et al. (*1*). To build our intersection database we have posed the following conditions: *a*) the chemical had to be a positive carcinogen according to the database of Gold et al. (*8*); *b*) the chemical had to be positive, or negative, in at least three tests and in at least 75% of the cases, to be defined as genotoxic or nongenotoxic. From our intersection database we found 113 chemicals that satisfied both conditions (Table 1).

Treating the database of Tennant et al. (*1*) in a similar way, we found 36 chemicals that satisfied conditions *a* and *b* (Table 2). Our database includes only 4 out of the 36 chemicals of Tennant's database (1,2-dibromo-3-chloropropane, 1,2-dibromoethane, di(2-ethylhexyl)phthalate, 1-phenylazo-2-naphthol).

For carcinogenic potency, Tennant gives the maximum tolerated dose that was administered, wheras Gold gives the TD_{50}. It was easy to extrapolate a TD_{50} for the data of Tennant. Using the positive carcinogens in common (32 chemicals) between the databases of Gold et al. (*8*) and Tennant et al. (*1*) (492 and 44 carcinogens, respectively), we correlated the maximum tolerated dose of Tennant and the TD_{50} of Gold. The equation of the regression line linking the two variables is $y = 0.04 + 1.06x$, where $y = \log_{10}(TD_{50})$ and $x = \log_{10}$(maximum tolerated dose). The correlation between the two parameters was good ($r = 0.97$ for 32 compounds). Using this equation we could express (Tables 2 and 3) the carcinogenic potencies of Tennant in terms of TD_{50}.

The database concerning our 113 compounds is shown in Table 1. The compounds have a roughly log-normal distribution, as expected (*10*).

In our database, we compared the carcinogenic potency of genotoxic and nongenotoxic carcinogens. The results obtained are shown in Table 3. Table 3 clearly shows that genotoxic compounds are more potent than nongenotoxic ones. Genotoxic compunds are about 50 times more potent for information coming from at least three tests (Fig. 1) and about 100 times more potent for information coming from at least six tests (Fig. 2). In both cases the differences are statistically significant. ($p < 0.0005$).

To get an idea of the importance of the difference, we can consider the following: In Figure 1, only about 8.2% of nongenotoxic carcinogens falls into the half to the left of the log-normal distribution of genotoxic carcinogens (the 50% most potent ones). This is true if at least three short-term tests are considered. If at least six short-term tests are considered (Fig. 2), then the 8.2% is reduced to 5.2% (a ratio of approximately 10 to 1 between the most potent genotoxic and the most potent nongenotoxic carcinogens).

Table 2. Database of Tennant et al. (*1*).

CAS number	Chemical name	$Log_{10}(TD_{50})$[a]
Genotoxic chemicals		
57-06-7	Allyl isothiocyanate	1.36
75-56-9	Propylene oxide	1.83
78-87-5	1,2-Dichloropropane	2.10
96-12-8	1,2-Dibromo-3-chloropropane	−0.51
101-80-4	4'-4'-Oxydianiline	0.99
101-90-6	Diglycidyl resorcinol ether	1.03
106-93-4	1,2-Dibromoethane	0.68
108-60-1	*bis*(2-Chloro-1-methylethyl)ether	2.00
137-30-4	Ziram	1.50
140-88-5	Ethyl acrylate	2.00
542-75-6	1,3-Dichloropropene	1.45
563-47-3	3'-Chloro-2-methylpropene	2.00
597-25-1	Dimethyl morpholinophosphoramidate	2.83
609-20-1	2,6-Dichloro-*p*-phenylenediamine	2.75
842-07-9	C.I. solvent yellow 14	1.41
868-85-9	Dimethyl hydrogen phosphite	2.32
2185-92-4	2-Biphenylamine 2HCl	2.78
2784-94-3	HC blue 1	2.34
2832-40-8	C.I. disperse yellow 3	2.47
2835-39-4	Allyl isovalerate	1.78
7446-34-6	Selenium sulfide	1.28
13552-44-8	4,4'-Methylenedianiline 2HCl	1.03
21739-91-3	Cytembena	0.55
26471-62-5	2,4- and 2,6-Toluene diisocyanate	1.77
Nongenotoxic chemicals		
50-55-5	Reserpine	−0.70
71-43-2	Benzene	1.36
78-42-2	*tris*(2-Ethylhexyl)phosphate	3.06
85-68-7	Butyl benzyl phthalate	2.98
87-29-6	Cinnamyl anthranilate	3.30
108-78-1	Melamine	2.43
117-81-7	di(2-Ethylhexyl)phthalate	2.66
140-11-4	Benzyl acetate	2.74
1746-01-6	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	−5.26
2432-99-7	11-Aminoundecanoic acid	2.67
5160-02-1	FD&C red no. 9	1.73
67774-32-7	Polybrominated biphenyl mixture	−0.70

[a] $Log_{10}(TD_{50})$ calculated according to the equation $y = 0.04 + 1.06x$, where $y = \log_{10}(TD_{50})$ and $x = \log_{10}$ (maximum tolerated dose), as explained in the text.

Table 3. Carcinogenic potencies of chemicals positive or negative in at least three tests and at least 75% of available tests.

	No. of chemicals	Mean value[a] (SE)	Median value[a] (I–III quartile range)
Intersection database of Gold et al. (*8*) and Würgler (*7*)			
Genotoxic	67	1.19 (1.44)	1.31 (0.246–6.03)
Nongenotoxic	46	52.5 (1.49)	64.1 (8.51–355)
Genotoxic[b]	39	0.53 (1.63)	0.904 (0.105–3.63)
Nongenotoxic[b]	42	52.2 (1.55)	49.5 (7.76–380)
Database of Tennant et al. (*1*)			
Genotoxic	24	45.3 (1.45)	60.0 (12.3–185)
Nongenotoxic	12	22.7 (5.24)	350 (0.653–834)

[a] The values are reported as TD_{50}, defined as "that chronic dose rate (in mg/kg body weight/day) which would halve the actuarially adjusted percentage of tumor-free animals at the end of a standard experiment time (the 'standard lifespan for the species')" (*9*). The original means and SE were computed on Log TD_{50} because we are dealing with a log-normal distribution: the mean should be multiplied or divided by SE (geometrical mean).

[b] Genotoxic or nongenotoxic carcinogens positive or negative in at least six tests and at least 75% of available tests.

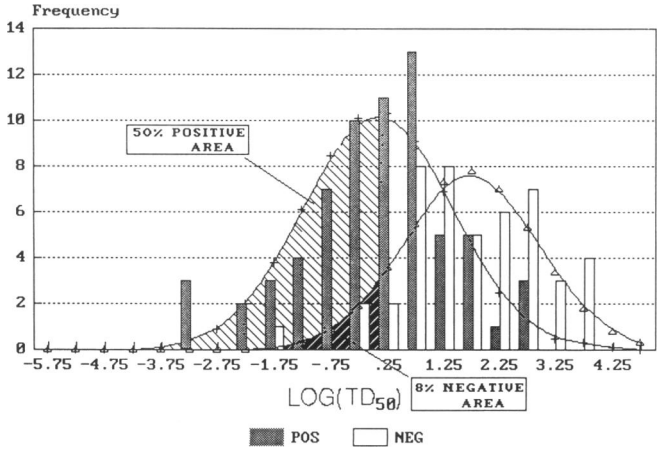

FIGURE 1. Distribution of log(TD_{50}) for genotoxic (shaded bars) and nongenotoxic (open bars) carcinogens positive or negative in at least three tests and at least 75% of the tests.

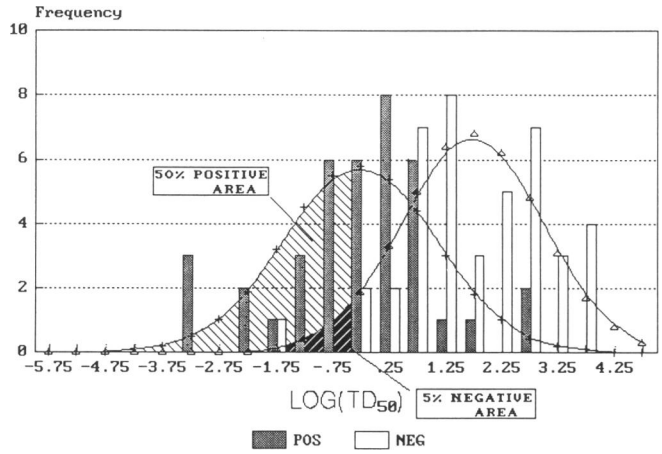

FIGURE 2. Distribution of log(TD_{50}) for genotoxic (shaded bars) and nongenotoxic (open bars) carcinogens positive or negative in at least six test and at least 75% of the tests.

We have treated the database of Tennant et al. (*1*) in the same way as our database. We select 36 chemicals from Tennant's database satisfying our conditions *a* and *b*. The database we obtained is summarized in Table 2. In this case genotoxic compounds are only 5.3 times more potent than nongenotoxic compounds in terms of median value; there is practically no difference when means are compared, especially as a consequence of the extremely high potency of TCDD (see Table 3).

The behavior of Tennant's database is essentially the behavior of all the compounds of the U.S. NTP database. From the analysis of Brown and Ashby (*11*), genotoxic carcinogens and nongenotoxic carcinogens in the NTP database, defined for their response in Salmonella and for structural alert, have a similar distribution in terms of range of potencies, only with a larger range of potency values for nongenotoxic carcinogens. In contrast, in the Gold-Würgler's database (Figs. 1 and 2), genotoxic carcinogens are clearly more abundant in the highest potency range, and nongenotoxic carcinogens are more abundant in the lowest potency range.

As suggested by Tennant et al. (*1*), compounds obtained from the literature can be affected by serious bias. One possibility, for instance, is that mainly genotoxic compounds were used for subsequent carcinogenicity studies. This, however does not seem to be the case for our database. In our database of 113 chemicals, we have 67 genotoxic compounds (59%) and 46 nongenotoxic compounds (41%). In the data base of Tennant et al. there are 24 genotoxic compounds (67%) and 12 nongenotoxic compounds (33%).

A second possible bias is represented by the fact that we could have started from a database much richer in carcinogens than the database of Tennant et al. (*1*) for our long-term studies in rodents. However, from the database of Gold et al. (*4–6*), 492 compounds can be defined as positive carcinogens (~ 50%) and 483 compounds as doubtful or negative carcinogens (~ 50%). From the global database of Tennant et al., 44 chemicals (~ 60%) can be defined as positive carcinogens and 29 chemicals (~ 40%) as doubtful or noncarcinogens.

The chemicals in Tennant's database were studied in a blind fashion; this is not the case for our database. Here we have two possibilities: *a*) a given chemical was already known to be a genotoxic agent. It is difficult in this case to envisage how this could have caused a bias by increasing the potency in the outcome of long-term experiments in rodents; *b*) the chemical was already known to be a carcinogen. If this caused a bias of heavily favoring the publication of positive data in terms of genotoxicity, most of the carcinogens of our database would be genotoxic, but we do not have a higher proportion of genotoxic carcinogens than Tennant et al. In conclusion, in our opinion, the fact that the chemicals of Tennant's database were tested blind cannot explain the discrepancies with our database.

We have also assessed whether the fact that in Würgler's database a much larger spectrum of short-term tests is considered than in Tennant's database could be partly responsible for the difference found between the two databases. For this purpose, to improve the correspondence between the short-term tests considered by us and Tennant we have used the pooled results obtained in Salmonella as a single test; considered the pooled results for sister chromatid exchanges as a single test; considered mammalian cytogenetics *in vitro* as a single test, and finally, used the mutagenicity data in mouse lymphoma L5178Y cells. With this restriction in the spectrum of short-term tests, we could define only 19 chemicals as genotoxic and only 5 chemicals as nongenotoxic. Genotoxic chemicals appeared, however, 192 times more potent than nongenotoxic ones.

To further explore the reasons for the observed differences we examined our intersection database and that of Tennant in terms of different chemical classes (Fig. 3). The histogram in Figure 3 clearly shows that the two databases are rather different nitrosocompounds, nitrogen mustards, hydrazine derivatives, and polycyclic aromatic hydrocarbons are absent in Tennant's database and nitrocompounds, alcohols, and phenols are heavily underepresented. Esters and carbamates, aromatic and heterocyclic amines and amides, and halogenated aromatics are more abundant (in terms of percentage) in Tennant's database. These differences in terms of chemical classes are probably sufficient to explain most of the differences in behavior of the Würgler-Gold database versus the Tennant database. If nitrosocompounds, nitrogen mustards, and hydrazine derivatives are excluded from the Würgler-Gold database, the difference in potency between genotoxic and nongenotoxic carcinogens is reduced roughly by half (Table 4). Even more important, if only

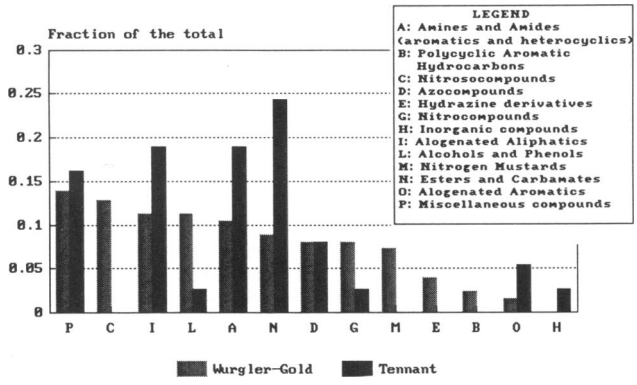

FIGURE 3. Subdivision of chemicals by chemical class in the database of Würgler-Gold (*7,8*) and in the database of Tennant (*1*). If more than one characterizing group was present in a given chemical, that chemical was placed in more than one chemical class.

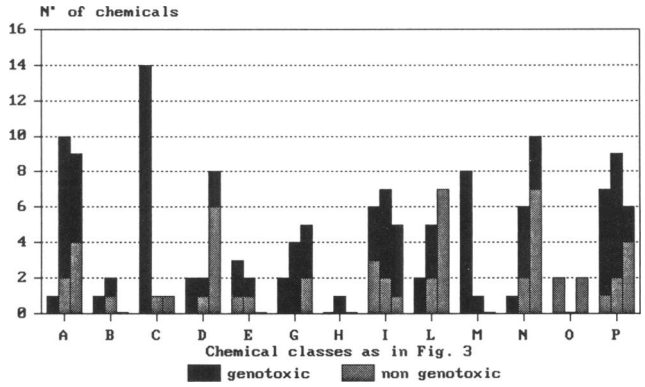

FIGURE 4. The databases of Würgler-Gold (*7,8*) and Tennant (*1*) were fused. A database of 141 chemicals (according to our definition of genotoxic or nongenotoxic carcinogens) was obtained. The 47 most potent chemicals are the far left columns of each group of three columns; the 47 chemicals of intermediate potency are the central columns, and the 47 least potent chemicals are the far right columns.

esters and carbamates, aromatic and heterocyclic amines and amides, and halogenated aromatics (the predominant classes in Tennant's database) are considered in the Würgler-Gold databases, than genotoxic carcinogens are only five times more potent than nongenotoxic ones (Table 4).

At this point, our results force the question of the representativity of different databases. We have no reason for considering our database more or less representative than Tennant's. Our database (113 chemicals) includes only 4 chemicals in Tennant's database and is therefore clearly of a different nature. Perhaps the truth is located somewhere in between the situation depicted by our database and that depicted by the database of Tennant and varies from chemical class to chemical class.

We also investigated whether some chemical classes show a definite trend, in the sense that they were especially rich in potent or weak carcinogens. For this purpose we assembled the database of Würgler-Gold and Tennant. We obtained a set of 141 different chemicals satisfying our definitions for genotoxic or nongenotoxic carcinogens. We subdivided this larger set in three parts: 47 most potent carcinogens, 47 average carcinogens and 47 weak carcinogens. The distribution in these three subsets for each chemical class is given in Figure 4.

Looking at Figure 4, some trends become apparent. Aromatic and etherocyclic amines and amides, azocompounds, nitrocompounds, alcohols and phenols, esters and carbamates, show a clear prevalence in the subclass of weak carcinogens. On the contrary, nitrosocompounds, hydrazine derivatives, and nitrogen mustards are clearly preferentially represented in the subclass of the most potent carcinogens.

In addition, we can also observe a general trend of enrichement in genotoxic carcinogens, going from the subset of weak carcinogens to the subset of potent carcinogens. Halogenated aliphatics and halogenated aromatics could be an exception to this general trend. Halogenated aromatics are all nongenotoxic in our database, and for several halogenated aliphatics promoting activities were found more relevant than initiating activities in the rat liver model of carcinogenesis (*13*). Obviously, the relevance of these considerations would be clearly increased if, in the future, we could deal with significantly larger sets of data, less subject to statistical fluctuations.

Discussion and Conclusions

We believe these data are important because we believe that the image offered by the work of Tennant (*1*) (and, as a consequence, the relevance that should implicitly be given to short-term tests for genotoxicity) should be re-equilibrated to a significant extent. Our results are complementary with what has been reported by Bartsch and Malaveille (*12*). Among the agents that

Table 4. Carcinogenic potency of genotoxic and nongenotoxic chemicals for different groups of chemical classes.

	Number of chemicals	Mean value,[a] TD_{50} (SE)	Ratio −/+[b]	Number of chemicals	Mean value,[c] TD_{50} (SE)	Ratio −/+	Number of chemicals	Mean value,[d] TD_{50} (SE)	Ratio −/+
Genotoxic[e]	67	1.19 (1.44)		40	3.14 (1.65)		23	8.83 (1.73)	
			44			20			4.6
Nongenotoxic[e]	46	52.5 (1.49)		41	64.1 (1.54)		16	40.4 (2.00)	
Genotoxic[f]	39	0.533 (1.63)		22	1.30 (2.12)		13	3.52 (3.10)	
			98			47			11
Nongenotoxic[f]	42	52.2 (1.55)		38	61.5 (1.60)		16	40.4 (2.00)	

[a]All chemicals of the Würgler-Gold intersection database.
[b]Nongenotoxic/genotoxic.
[c]Nitrosocompounds, nitrogen mustards, hydrazine derivatives, and polycyclic aromatic hydrocarbons excluded.
[d]Only esters, carbamates, aromatic and heterocyclic amines and amides, and halogenated aromatics included.
[e]Genotoxic or nongenotoxic carcinogens positive or negative in at least three tests and at least 75% of available tests.
[f]Genotoxic or nongenotoxic carcinogens positive or negative in at least six tests and at least 75% of available tests.

have been included in the *IARC Monographs* (agents to which humans are currently exposed) and that have been considered to be carcinogenic, probably carcinogenic, or possibly carcinogenic to humans, there is a high prevalence (80–90%) of genotoxic carcinogens (*12*). This result, like ours, stresses the importance of genotoxic carcinogens. After submitting this manuscript, we were informed by L. S. Gold (personal communication) that our observation holds also when looking only at mutagenicity in Salmonella: "more toxic carcinogens are significantly more likely to be mutagenic than less toxic carcinogens" (*14*). In addition, going from a highest daily dose of less than 1 mg/kg/day to a highest daily dose of more than 1000 mg/kg/day, the fraction of carcinogens mutagenic in Salmonella decreases regularly from 71–76% to 28–13% in mice and rats, respectively (L. S. Gold, personal communication).

The compounds in our present analysis have been considered (both for carcinogenicity and genotoxicity) only as tested at high subtoxic dosages. The question is what will the extrapolation of potencies at lower doses be? Genotoxic carcinogens could have a more linear or less sublinear extrapolation at low doses than nongenotoxic carcinogens. In this case, the difference in potency between genotoxic and nongenotoxic carcinogens at doses relevant to human exposure could be even greater than the degree shown in this report. We do not know if the globality of carcinogenicity experiments, with genotoxic or nongenotoxic carcinogens, respectively, tends to show a systematic difference in the dose–response curve. Perhaps this could be an interesting field for future investigations.

Promoting and epigenetic effects are probably relevant carcinogenicity components both in rodents and in humans. Little is known about the extrapolability from rodents to humans of these kinds of effects. It is not even known if short-term *in vitro* tests for these types of effects will become technically possible in the near future. At present, we can most likely protect ourselves much better from the genotoxic component of carcinogenicity rather than from the promotion–epigenetic component. It is therefore important that studies to better assess the relevance to humans of nongenotoxic carcinogens be more thoroughly developed.

It seems, however, that for a completely new chemical, short-term genotoxicity tests perform a useful task; not only do they inform us about one of the two major components of the carcinogenetic process (irreversible alterations in the genome), they also tend to detect a fraction of rather potent carcinogens. There are nongenotoxic carcinogens such as TCDD that are very potent, but this type of epigenetic carcinogen is apparently rare. For a noncovalent interaction to induce a potent effect, a high affinity to a specific cellular receptor is expected. From a probabilistic point of view, for a molecule unrelated to the conformation of the receptor itself, having good complementarity with a cellular receptor should be a rare event (*12*).

Our results seem to modify the impression offered by the results of the work published by Tennant and co-workers (*1*). They seem to suggest that, even if short-term genotoxicity tests are not very good predictors of carcinogenicity in rodents because they can detect only a fraction of the factors that are relevant for the process of carcinogenesis, they are still useful because they tend to detect (as an average) the most potent carcinogens. This is equivalent to saying that even if irreversible alterations in the genome (genotoxic effects) are not the only component, they are still a very important (often the most important) component of the process of chemical carcinogenesis.

We are grateful to J. Ashby, H. Bartsch, R. Beningni, N. Loprieno, and F. Würgler for their discussion of the manuscript and their useful suggestions. We are grateful to Miss G. Frigerio for careful typing of the manuscript. This work was supported by grants from the European Community [EV-4V 0036-I(A)] and from the Italian Ministry of University, Scientific and Technological Research (Project IST-CIRC-AN-SALDO).

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